

Retention of Co 60 Labeled Vitamin B₁₂ in Chickens.* (20313)

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It has been reported from this laboratory (1) that injection of crystalline vit. B₁₂ into eggs, deficient in this vitamin, results in a growth response in the chicks hatched from these eggs which is sustained for at least 12 weeks. This raises the question as to whether this prolonged growth response is due to an initial growth impetus or to the retention of the vitamin throughout the experimental period.

The present work was planned to determine whether or not a significant quantity of vit. B₁₂ was retained in the chick over a 12-week period when the Co 60 labeled vitamin was administered by injection into vit. B₁₂-deficient eggs.

Experimental. Eggs used for injection were from Rhode Island Red hens fed a vit. B₁₂-deficient diet. The eggs were injected on the 6th day of incubation as follows: One lot was injected with 3 μ g of Co 60 labeled vit. B₁₂ which had a specific activity of about 38 μ c per mg; a second lot was injected with 3 μ g of the unlabeled vitamin (Cobione); a third lot was not injected. Chickens hatched from all the eggs received a corn-soybean meal diet which was low in vit. B₁₂. At least 5 birds from each group were sacrificed at one day, 3, 6, 9, and 12 weeks of age. The chickens from the B₁₂-injected groups demonstrated a growth response similar to that obtained by Lillie *et al.*(1). **Radioactivity measurements.** Individual, whole chickens were wet ashed, made to volume and their radioactivity determined with a proportional counter. All samples were counted for a sufficient length of time to insure a counting error of not more than 5%. All results were referred to a standard prepared in the same manner as the samples. Self absorption and decay correc-

tions were applied in all cases. The samples were prepared for counting by direct evaporation of the nitric acid digests in stainless steel cups. Twenty chicks from the radioactive group were kept in a separate battery and the droppings collected quantitatively for each of the 12 weeks of the experimental period. Each weekly collection of droppings was dried, ground and a representative sample wet ashed and made to volume. **Microbiological assay.** The B₁₂ content of chickens from the eggs injected with Cobione was estimated by the U. S. Pharmacopeia method(2), using cyanide in the extraction procedure(3). In order to measure only the B₁₂ content in the tissues of the chicken, the intestinal contents were discarded. The chickens were skinned in order to facilitate easy removal of the feathers.

Results and discussion. The results of radioactivity measurements and microbiological assays on whole chicks for the various age groups are reported in Table I. The vit. B₁₂ values of the measured radioactivity are reported, assuming that there was no degradation of the vitamin after injection. In support of this assumption Rosenblum and coworkers (4) have concluded, on the basis of parallel radioactivity and microbiological assays, that the injected radiovitamin is excreted as such in the urine of the rat.

The results indicate that there was a rapid initial loss of radioactivity during the first 3 weeks, after which there was a rather uniform and continuous loss for the remainder of the experimental period. Monroe and coworkers(5) have reported, on the basis of a short term experiment, that B₁₂ has a low turnover rate in week-old chicks when the vitamin is injected intraperitoneally. The results reported here confirm this low turnover rate for B₁₂. In addition, the data show that as much as 30% of the injected vitamin is retained by the chick over a 12-week period.

The vit. B₁₂ values for the droppings and

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TABLE I. Vitamin B₁₂ Values for Chicks and Droppings Estimated by Microbiological Assay and Calculated from Radioactivity Measurements.

Age	B ₁₂ values estimated by microbiologi- cal assay, μ g	—B ₁₂ values calculated from radioactivity—		
		Per chick, μ g	Cumulative excretion per chick, μ g	% recovery
(Egg)	3.12*	3.0	—	100.0
1 day	3.03*	2.46	—	—
3 wk	1.20	1.71	.94	88.5
6 "	1.61	1.42	1.65	102.2
9 "	2.18	1.23	—	—
12 "	4.10	.98	—	—

* 3 μ g inj. B₁₂ + B₁₂ normally present.

the percent recovery as determined by adding the total B₁₂ excreted to that retained by the chicks are shown in the last 2 columns in Table I. Essentially complete recovery of the radioactivity was obtained for the 3- and 6-week intervals. Although droppings were collected weekly for the entire experimental period, values are reported only for the first 6 weeks because of the large dilution of the small amount of radioactivity in the later samples.

A comparison of the values reported, based on the 2-assay methods, suggest that there was no appreciable uptake of the vitamin from the basal diet or intestinal synthesis during the first 6 weeks of the experiment, since the data are in approximate agreement for these age groups. Beyond 6 weeks of age there is a decided increase in the B₁₂ values based on microbiological assay compared to those calculated from radioactivity, indicating absorption of the vitamin from some exogenous source. The difference in the chick B₁₂ values noted at 3 weeks is of doubtful significance and probably represents experi-

mental error in one or both of the assay methods. However, the possibility that this difference represents Co 60 in some form other than vit. B₁₂ can not be ruled out.

Summary. The results indicate that over 30% of the injected Co 60 activity is retained for as long as 12 weeks in chicks hatched from eggs injected with the radio-vitamin. The total B₁₂ content of the chick as measured microbiologically roughly parallels Co 60 activity until 6 weeks of age, after which there is a decided increase in the total B₁₂ content of the chicks.

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